# Silicon-Mediated Resilience: Unveiling the Protective Role against Combined Cypermethrin and Hymexazol Phytotoxicity in Tomato Seedlings

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## Abstract

Insecticides and fungicides present potential threats to non-target crops, yet our comprehension of their combined phytotoxicity to plants is limited. Silicon (Si) has been acknowledged for its ability to induce crop tolerance to xenobiotic stresses. However, the specific role of Si in alleviating the cypermethrin (CYP) and hymexazol (HML) combined stress has not been thoroughly explored. This study aims to assess the effectiveness of Si in alleviating phytotoxic effects and elucidating the associated mechanisms of CYP and/or HML in tomato seedlings. The findings demonstrated that, compared to exposure to CYP or HML alone, the simultaneous exposure of CYP and HML significantly impeded seedling growth, resulting in more pronounced phytotoxic effects in tomato seedlings. Additionally, CYP and/or HML exposures diminished the content of photosynthetic pigments and induced oxidative stress in tomato seedlings. Pesticide exposure heightened the activity of both antioxidant and detoxification enzymes, increased proline and phenolic accumulation, and reduced thiols and ascorbate content in tomato seedlings. Applying Si (1 mM) to CYP- and/or HML-stressed seedlings alleviated pigment inhibition and oxidative damage by enhancing the activity of the pesticide metabolism system and secondary metabolism enzymes. Furthermore, Si stimulated the phenylpropanoid pathway by boosting phenylalanine ammonia-lyase activity, as confirmed by the increased total phenolic content. Interestingly, the application of Si enhanced the thiols profile, emphasizing its crucial role in pesticide detoxification in plants. In conclusion, these results suggest that externally applying Si significantly alleviates the physio-biochemical level in tomato seedlings exposed to a combination of pesticides, introducing innovative strategies for fostering a sustainable agroecosystem.

**Keywords:** pesticides; silicon; secondary metabolism; co-exposure; detoxification system; tomato.

## 1. Introduction

In contemporary agricultural ecosystems, plants inevitably confront diverse forms of abiotic stress, including combined pesticide exposure driven by their cost-effectiveness and high efficiency (Sarker et al., 2023). Consequently, these pesticides are extensively and indiscriminately used to manage crop diseases and control agricultural pests (Mahapatra et al., 2019). Algeria has witnessed a significant increase in the use of agrochemicals in response to a growing national demand for cereals and fruits. In Benchicao, a region recognized for mountain-intensive viticulture and agriculture in Medea province of Algeria, the systemic fungicide hymexazol is extensively employed for its effective control of fungal diseases, such as fusarium, Pythium, and *Rhizoctonia solani*, across a variety of crops (Fan et al., 2017; Hassanen et al., 2022). On the other hand, pyrethroid insecticides, particularly cypermethrin (CYP), hold global significance due to their widespread applications and high efficacy against various agricultural pests. CYP, the most commonly used synthetic pyrethroid insecticide, binds to acetylcholine receptors, inducing neuronal shocks that lead to paralysis and death in insects (Kumar et al., 2023).

Pesticide exposure, including harmful xenobiotic chemicals, can result in severe morpho-physiological and biochemical abnormalities in non-target plant crops (Alves et al., 2023; D. Li et al., 2023; Zhang et al., 2022). These pesticides enter the soil through soil amendments and foliar applications, leading to residual persistence, soil pollution, and food web contamination, with potential consequences for non-target soil organisms (Kumar et al., 2023; Ullah et al., 2018). Residual pesticides deposited in the soil may be absorbed by plant roots, affecting various plant development processes at the morphological, physiological, biochemical, and molecular levels (Zhang and Li, 2023). Physio-biochemical disorders induced by pesticides in plants are predominantly associated with increased oxidative stress (Soares et al., 2023; Zhang et al., 2022).

However, plants have evolved diverse biochemical strategies to tolerate pesticide exposure, including enzymatic and non-enzymatic detoxification (Soares et al., 2019a).

Pesticide metabolism in plants generally involves three-phase detoxification pathways (Xia et al., 2009): (i) consisting of redox and/or hydrolysis reactions catalyzed by enzymes like cytochrome P450 monooxygenase, carboxylesterase, and peroxidase (Yu et al., 2022); (ii) involving the conjugation of Phase I metabolites with glucose and reduced glutathione catalyzed by UDP glycosyltransferase and glutathione-S-transferase (GST) (Peng et al., 2023a); and (iii) encompassing the transport of low-toxicity conjugates into vacuoles bound to cell walls  (Ahammed et al., 2013; Peng et al., 2023a). Certain plant growth regulators (silicon) and hormones (brassinosteroid and melatonin) have been reported to play a role in regulating plant pesticide detoxification processes (Peng et al., 2023a; Sharma et al., 2019b; Soares et al., 2021). Recently, the co-contamination of pesticides has gained attention as an ecotoxicological hazard, demonstrating increased phytotoxicity and altered biological activities that impact plant growth (Alves et al., 2023). Therefore, it is crucial to investigate the adverse effects of pesticides, both individually and in combination, on non-target crops and develop effective strategies to alleviate pesticide toxicity in non-target plants.

Various approaches have been employed to minimize pesticide-induced phytotoxicity, including applying signaling molecules and phytohormones and breeding crop varieties resistant to xenobiotics (Kumar et al., 2023). Among these, nutrient supplementation has proven effective in promoting plant growth and reducing xenobiotic phytotoxicity. Silicon (Si), although not an essential element for plants, has been increasingly recognized for its ability to alleviate abiotic stress. Studies have reported beneficial effects of Si on plant biomass, photosynthesis, gas exchange, nutrient uptake, seed germination, and the antioxidant defense system in different plant species (Calzada et al., 2023; He et al., 2023; Improvement, 2022). Additionally, Si application has been shown to mitigate the phytotoxic impact of fungicide toxicity in wheat (Tripthi et al., 2020). Previous research has demonstrated that supplying Si to tomato plants exposed to glyphosate prevented the herbicide's phytotoxic effects

(Soares et al., 2021). In our previous study, Si significantly reduced the combined phytotoxic effects of salt and insecticides, enhancing photosynthetic pigments and the antioxidant defense system in tomato seedlings (Touzout, 2023). However, further investigation requires a deeper understanding of the regulation of antioxidative, thiol, and secondary metabolism pathways by Si to reduce combined pesticide phytotoxicity.

This research explores the potential mitigating effects of Si against phytotoxic injuries induced by CYP and/or hymexazol (HML). The focus of the current study was i) to investigate the morpho-physio-biochemical changes of tomato seedlings in response to CYP and HML stress, ii) to examine the effects of Si and pesticide treatments on seedling growth, photosynthetic pigments, oxidative damage, antioxidant system, and secondary metabolism in tomato seedlings, and iii) to explore the involvement of Si in the metabolism and detoxification of CYP and HML in tomato seedlings. It is noteworthy that, to date, a dearth of research exploring the protective effects of Si against phytotoxic injuries induced by the combination of CYP, HML, and their exposures. Importantly, this pioneering research investigates the specific protective effects of Si under combined exposure to CYP and HML, opening new avenues and expanding our understanding of interactions between these compounds. The novelty of this study lies in its exploration of the potential of Si as a protective agent against the phytotoxic injuries induced by CYP and/or HML.

## 2. Materials and methods

### 2.1. Chemicals

Data pertaining to pesticide usage in Medea was systematically acquired through a well-crafted survey administered to local farmers. The study focused on fungicides and insecticides commonly employed in viticulture and arboriculture, the predominant crops in Benchicao (Medea region, South-West Algeria, Latitude 36°

1154.2900N, Longitude 2 500 43.4200E), a mountainous region at an elevation of 1000 m.

Two commercially available pesticides extensively used in viticulture were chosen: the fungicide hymexazol (Tachigazole SL - active ingredient (a.i.) hymexazol – HML, 30%) and the insecticide pyrethroid (Sherpa 25 EC, (a.i.) cypermethrin – CYP, 10.8%), both procured from Syngenta Crop Protection AG, Algeria. As specified by their respective manufacturers, the recommended field application rates stand at 1 L ha$^{-1}$ for Tachigazole and 1.5 L ha$^{-1}$ for Sherpa. To maintain precision, these pesticides were freshly prepared in deionized distilled water, adhering to the required concentration of the active ingredient. All other reagents utilized in the study met the standards of analytical grade.

## 2.2. Plant species and growth conditions

The tomato (*Solanum lycopersicum* L.) cultivar Doucen seeds were obtained from the Technical Institute for Market and Industrial Crops (ITCMI Staouali, Algiers). A meticulous disinfection process was employed to ensure seed purity, involving a 10-minute immersion in a 0.5% v/v sodium hypochlorite solution, followed by thorough rinsing in deionized water. The selected seeds, exhibiting uniformity and good health, were germinated in a petri dish covered with moist double-layered filter paper within a dark incubator, maintaining a temperature of 27°C for 7 days.

For the hydroponic phase, tomato seedlings with comparable growth characteristics were transplanted into a box containing a full-strength nutrient solution Hoagland (pH 6.0 ± 0.5). The growth chamber conditions were controlled, maintaining a 14-h photoperiod at a temperature of 25 ± 2 °C, a photosynthetic photon flux density (PPFD) of 600 μmol m$^{-2}$ s$^{-1}$, and 70% relative humidity (Touzout, 2023). Following a 15-day acclimatization period in the nutrient solution, the tomato seedlings were exposed to pesticide stress and Si treatments. The experimental design was completely randomized design (CRD) encompassed eight treatments each was replicated three times: T1: CK control (Hoagland solution and sprayed with tap water); T2: Si ($Na_2SiO_3$; 1 mM added to Hoagland solution); T3: CYP (seedlings were sprayed with the recommended dose (1.5 L $ha^{-1}$); T4: Si + CYP (1 mM Si, 1.5 L $ha^{-1}$ CYP); T5: HML (seedlings were sprayed with the recommended dose (1 L $ha^{-1}$); T6: Si + HML (1 mM Si, 1 L $ha^{-1}$ HML); T7: CYP + HML (1.5 L $ha^{-1}$ CYP, 1 L $ha^{-1}$ HML); T8: Si + NaCl + VOL (1 mM Si, 1.5 L $ha^{-1}$ CYP, 1 L $ha^{-1}$ HML). The Si concentration was chosen based on our previous results (Touzout, 2023). In addition, 1 mM Si application demonstrated enhanced tolerance of *Phoenix dactylifera* plants under combined cadmium and salinity stress (Khan et al., 2020). The selection of the insecticides and fungicides was guided by a survey conducted in Benchicao, Medea province 2022, indicating their prevalent use against viticulture and arboriculture pests. The concentrations of CYP and HML applied adhered to their respective manufacturers' recommended field application rates. The nutrient solution (pH 6.0 ± 0.5) underwent regular aeration and refreshing every two days to prevent nutrient depletion. Following a 7-day exposure period, the seedlings were harvested to collect comprehensive data on various morpho-physiological and biochemical attributes in tomato seedlings.

### 2.3. Determination of Growth attributes

Tomato seedlings were carefully divided into shoots and roots, receiving a thorough wash with deionized water, followed by gentle drying using filter paper. The lengths of both shoots and roots were then measured using a ruler, and their respective fresh weights were accurately assessed using a digital analytical balance. To record dry weights, seedlings were dried in an oven for 24 at 80 °C h.

### 2.4. Determination of photosynthetic pigments

The quantification of chlorophyll and carotenoid contents adhered to the method described (Arnon, 1949). Fresh leaves underwent extraction in darkness using an 85% (v/v) acetone solution at 4 °C for 24 hours. Absorbance readings were recorded at 650 nm for chlorophyll a, 665 nm for chlorophyll b, and 470 nm for carotenoids. The concentrations of these pigments were then computed following the procedure outlined by (Lichtenthaler, 1987).

### 2.5. Determination of oxidative stress biomarker

Tomato leaves with a mass of 0.1 g were ice-homogenized in 3 mL of a 0.1% trichloroacetic acid solution. The supernatant was collected after centrifugation at 4 °C for 15 minutes at 12000 g. The quantification of hydrogen peroxide ($H_2O_2$) and lipid peroxidation (malondialdehyde, MDA content) levels followed established methods as documented in the literature (Heath and Packer, 1968; Velikova et al., 2000). $H_2O_2$ concentrations were determined using an extinction coefficient of 0.28 $\mu M^{-1}$ $cm^{-1}$, while MDA concentration was calculated based on an extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.

### 2.6. Determination of antioxidants and detoxification enzymes activities

Fresh leaves were homogenized in an ice-cold potassium phosphate buffer (100 mM, pH 7.8, containing 0.2 mM EDTA and 1% PVP) and then centrifuged at 15,000 g for 15 minutes at 4 °C. For APX extraction, 2 mM Ascorbic Acid (AsA) was incorporated. The resulting supernatant was employed to evaluate total protein content and enzyme activities. The total soluble protein content was determined by following the protocol recommended by (Bradford, 1976). Catalase (CAT) activity was assessed in accordance with the procedure outlined by (Aebi, 1984), while the measurement of Ascorbate peroxidase (APX) activity followed the method suggested by (Nakano and Asada, 1981). Spectrophotometric analyses were conducted for GST and peroxidase (POD) activities (Cakmak and Marschner, 1992; Habig and Jakoby, 1981). The phenylalanine ammonia lyase (PAL) methodology was executed following the guidelines provided by (Sánchez-Rodríguez et al., 2011).

### 2.7. Determination of thiols profile contents

The determination of reduced glutathione (GSH) levels involved the recycling method with 5,5-dithiobis (2-nitrobenzoic acid, DTNB), and the optical density of the samples was observed at 412 nm (Anderson, 1985). Fresh leaves (0.1 g) underwent homogenization in an ice bath with 20 mM EDTA (3 mL) and subsequent centrifugation at 4 °C for 20 minutes at 12,000 g. The quantification of Total Thiols (TT), Protein Thiols (PT), and Non-Protein Thiols (NPT) content was carried out by employing an extinction coefficient of 13.6 $mM^{-1}$ $cm^{-1}$, expressed as nmol per g fresh weight, following the protocol detailed by (Sedlak and Lindsay, 1968). PT values were obtained by subtracting NPT from TT (Sedlak and Lindsay, 1968).

### 2.8. Determination of Non-enzymatic antioxidants contents

The homogenization of leaf tissues (0.1 g) took place in 3% sulphosalicylic acid (3 mL), and the resulting homogenate was centrifuged at 4 °C for 15 minutes at 12000 g. Proline content was determined using the ninhydrin colorimetric assay, following the methodology outlined in (Bates et al., 1973). Total phenolic contents were measured according to the procedure described in (Singleton and Rossi, 1965), with the optical density recorded at 750 nm. Reduced ascorbate (AsA) levels were quantified from leaves, employing the protocol detailed in (Murshed et al., 2008).

### 2.9. Statistical analysis

The mean ± standard errors of three replicates were used to present the data. Statistical analysis included one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to compare treatment groups at a significance level of $p < 0.05$. IBM SPSS 22 (ver.22.0, SPSS Inc., Chicago, IL, USA) was employed for the statistical analysis, and GraphPad Prism 8 software was utilized for creating all plots. To visualize the data, generate principal component analysis (PCA), hierarchical cluster plot and pearson correlation analysis, the Origin software was used (OriginLab, 2021).

## 3. Results

### 3.1. The effects of Si supplementation on growth in pesticides-administrated tomatoes

Fig. 1A reveals a significant impediment to seedling growth due to pesticides exposure compared to the control group. Tomato seedlings exposed to CYP and/or HML displayed a decline in shoot biomass and length, with the most pronounced reduction observed in combined exposure (CYP + HML), showing a 31% decrease in shoot biomass and a 17% decrease in length (Figs. 1A and 1B). Under non-stress conditions (control), Si application had no discernible effect on seedling growth parameters, as shown in Fig. 1.

In the presence of pesticides, the exogenous application of Si significantly improved shoot biomass, whereas non-significantly improved shoot length in tomato seedlings when compared with respective alone pesticide treatment (Fig. 1A and 1B). Si significantly alleviated the stress caused by CYP, HML, and CYP+HML on tomato seedlings shoot biomass by 17%, 13%, and 15%, respectively, in comparison to seedlings stressed individually by CYP, HML, and CYP+HML without Si (Fig. 1A).

Likewise, root biomass and length reduction were observed in pesticides-exposed tomato seedlings (Figs. 1C and 1D). A more pronounced decline in root biomass (27%) and length (25%) occurred under combined stress treatment. In the presence of stress, exogenous Si application promoted the growth of tomato roots. Si application to CYP, HML, and CYP+HML stressed seedlings significantly improved root biomass by 4%, 17%, and 15%, respectively, and root length by 11%, 14%, and 12%, respectively, compared to seedlings stressed individually by CYP, HML, and CYP+HML (Figs. 1C and 1D). The Si-induced growth enhancement was statistically significant, except for Si+CYP in root biomass (Fig. 1D).

Exposure to CYP and/or HML was observed to decrease the dry weight of seedlings significantly compared to the control group (Fig. 1E). The inhibitory effects of CYP+HML on dry weight were much stronger than those of chemicals alone (Fig. 1E). Exogenous Si application to CYP, HML, and CYP+HML treated seedlings was able to enhance significantly the dry weight by 9, 11, and 18%, respectively, compared to seedlings treated only by CYP, HML, and CYP+HML (Fig. 1E).

### 3.2. The effects of Si supplementation on photosynthetic pigments in pesticides-administrated tomatoes

Figure 2 summarizes the individual and combined impacts of stress induced by insecticides and fungicides on photosynthetic pigments. A conspicuous reduction in chlorophyll (Chl a, Chl b) and carotenoid levels is observed under stress conditions compared to the control ($P < 0.05$). The most substantial decline in Chl a is noted under CYP (47%), succeeded by CYP+HML (38%) and HML (33%) in comparison to the control groups (Fig. 2A). Chl b content undergoes a reduction when tomato seedlings are subjected to both individual and combined pesticide exposures, exhibiting a noteworthy ($p < 0.05$) decrease by 46%, 41%, and 25% in CYP, HML, and CYP+HML, respectively, relative to the control groups (Fig. 2B). Similarly, a parallel pattern is observed for carotenoids following exposure to CYP and HML pesticides (Fig. 2C).

Contrarily, incorporating Si alongside CYP and/or HML significantly enhances photosynthetic pigments compared to treatments with CYP and/or HML alone. Specifically, Chl a content exhibits an increase of 58%, 19%, and 52% following Si+CYP, Si+HML, and Si+CYP+HML, respectively, compared with their treatments without Si. Likewise, Si tends to alleviate CYP and/or HML-induced decreases in tomato seedlings' Chl b and carotenoid levels (Figs. 2B and 2C).

These findings underscore the potential protective influence of Si against injuries induced by CYP and/or HML on photosynthetic pigments.

### 3.3. The effects of Si supplementation on oxidative stress biomarkers in pesticides-administrated tomatoes

In comparison to the untreated control, tomato leaves displayed a significant elevation in both $H_2O_2$ generation and MDA levels when exposed to CYP and/or HML, as shown in Fig. 3. The increase in $H_2O_2$ generation was pronounced, with CYP treatment leading to an approximately 111% rise, followed by 91% under CYP+HML, and 56% under HML, as compared to the control (Fig. 3B). Introducing Si to seedlings treated with CYP, HML, or CYP+HML resulted in a marked reduction in $H_2O_2$ levels compared to the respective treatments without Si. The reductions are 18%, 14%, and 9% following Si+CYP, Si+HML, and Si+CYP+HML, respectively as shown in Fig. 3B.

Similarly, MDA levels experienced a surge of 231% and 52%, respectively, compared to the untreated control, following exposure to CYP and HML. Notably, the combined exposure to CYP and HML yielded a diminished effect (115%) compared to the cumulative impact of the separate treatments, indicating an antagonistic combined effect of CYP and HML on lipid peroxidation (Fig. 3A). Interestingly, the application of Si significantly enhanced the tolerance of tomato seedlings to CYP, HML, and the combined CYP and HML stresses (Fig. 3A), resulting in notable reductions of 51%, 23%, and 42% in MDA levels compared to their respective treatments without Si. These results show that Si considerably alleviated the adverse effects of pesticides-induced oxidative damage.

### 3.4. The effects of Si supplementation on antioxidant and biotransformation enzymes activities in pesticides-administrated tomatoes

Exposure to pesticides resulted in a marked elevation ($p < 0.05$) in the activities of CAT and APX in the leaves of seedlings subjected to CYP and/or HML, as seen from Figs. 4A and 4B. Particularly noteworthy were the effects of the fungicide HML after 7 days, showing a 335% increase in CAT and a 306% increase in APX activities compared to the control group. The application of Si alongside CYP and HML reduced CAT and APX values compared to their individual exposures, as shown in Figs. 4A and 4B. Conversely, Si application in conjunction with CYP+HML significantly boosted CAT and APX by 64% and 27%, respectively, compared to treatments without Si (Figs. 4A and 4B).

In terms of detoxifying enzymes, the catalytic activity of GST significantly heightened ($p < 0.01$) when tomato seedlings were exposed to CYP, HML, and the combined exposure to CYP and HML in comparison to the control (Fig. 4C). Notably, the co-exposure of CYP and HML demonstrated a more pronounced induction of GST activity compared to seedlings stressed with pesticides individually, suggesting a synergistic effect on GST upregulation (Fig. 4C). Similarly, CYP, HML, and their combination induced POD activity compared to the control (Fig. 4D). However, the combined pollution had a less pronounced impact on the upregulation of POD activity, indicating an adverse effect on its activation.

While the addition of Si alone increased GST and POD activities compared to the untreated control (Fig. 4C and D), applying Si to CYP- and HML-stressed tomato seedlings significantly reduced the activity of detoxifying enzymes. There was a notable increase in POD activity in Si+CYP+HML treatment compared to their respective CYP+HML treatment (Figs. 4C and 4D), suggesting that adding Si may mitigate the stresses associated with pesticides.

### 3.5. The effects of Si supplementation on thiols profile in pesticides-administrated tomatoes

To explore Si's protective influence against damage caused by CYP and/or HML on the thiol profile, we assessed and compared thiol compound levels in Si-treated seedlings exposed to individual and combined CYP and HML stresses. The application of CYP and HML, separately or together, resulted in a substantial reduction in TTSH and PT levels, as seen in Figs. 5A and 5B. Particularly noteworthy was the significant decrease in PT content under HML (58%), followed by CYP+HML (39%) and CYP (36%) compared to the control groups (Fig. 5B). In contrast, Si supplementation significantly elevated PT content by 93%, 63%, and 79% in CYP, HML, and CYP+HML-exposed tomato seedlings, respectively, compared to those stressed solely by CYP, HML, and CYP+HML (Fig. 5B).

The measured leaf GSH content of tomato seedlings exposed to pesticides exhibited a reduction of 36%, 49%, and 39% following CYP, HML, and CYP+HML treatments, respectively, in comparison to the control group as shown in Fig. 5D. Remarkably, the application of Si reversed the observed decline in GSH content induced by CYP and/or HML exposures. Si supplementation to CYP, HML, and combined CYP and HML stressed tomato seedlings resulted in a significant increase in GSH content by 52%, 98%, and 99% following Si+CYP, Si+HML, and Si+CYP+HML treatments, respectively, compared to their respective pesticide single treatments (Fig. 5D). A similar trend was observed for NPT content as shown in Fig. 5C. Furthermore, CYP, HML, and their combination significantly reduced the NPT level compared to the control (Fig. 5C). Conversely, the application of Si effectively reversed the decrease in NPT content triggered by xenobiotic exposures (Fig. 5C).

### 3.6. The effects of Si supplementation on secondary metabolism and non-enzymatic antioxidants in pesticides-administrated tomatoes

The tomato seedlings, when subjected to individual and combined stresses of CYP, HML, and CYP+HML, displayed a notable increase in total phenolic content (TPC) compared to the control seedlings ($P < 0.01$) as shown in Fig. 6A. The most significant increments were observed in the cases of individual CYP and HML stresses (37% and 57%, respectively) when compared to the control. In contrast, the increase in seedlings under the combined stress condition was relatively lower (33%) than those exposed individually to CYP and HML stresses. Applying Si alone led to an increase in TPC compared to the control groups. However, the co-application of Si with CYP, HML, and CYP+HML did not induce any significant alteration in TPC accumulation, except for Si+HML, which notably increased the TPC content in tomato seedlings compared to their respective CYP, HML, and CYP+HML treatments.

Fig. 6B shows that PAL activity was stimulated by the presence of CYP and/or HML. Compared to the control group, PAL activity increased by 85%, 254%, and 85% following the application of CYP, HML, and CYP+HML stresses, respectively. Applying Si alone significantly elevated PAL activity compared to the control groups. Intriguingly, when Si was applied to CYP+HML-stressed tomato seedlings, PAL activity increased by 128% following Si+CYP+HML treatments compared with their respective single pollutant treatments.

Proline accumulation saw a significant increase of 26% and 35% during individual stresses of insecticide CYP and fungicide HML, respectively, compared to the control seedlings, as shown in Fig. 6C. The highest proline content (45%) was recorded under the combined treatment. Furthermore, applying Si alone or combined with CYP, HML, and CYP+HML further heightened proline content compared to the control or their respective individual treatments.

The level of AsA exhibited a significant increase ($P < 0.01$) when exposed to CYP, as shown in Fig. 6D. However, its level decreased ($P < 0.05$) in the case of HML alone and in combination with CYP compared to the control group. Interestingly, the level of AsA showed a significant decrease after Si application compared to the control. Nevertheless, applying Si to seedlings stressed with CYP and/or HML did not result in any marked change, except for the significant decrease observed in Si+CYP treatment compared to their treatments without Si.

### 3.7. Multivariate analysis

#### 3.7.1. Principal Component Analysis

The principal component analysis (PCA) of the plant response data revealed that the first principal component (SB) had an eigenvalue of 11.56528, accounting for 52.57% of the total variance. The second principal component (SL) had an eigenvalue of 4.53475, explaining an additional 20.61% of the variance, bringing the cumulative variance explained to 73.18%. The third principal component (RB) had an eigenvalue of 2.00017, contributing 9.09% to the total variance, with a cumulative variance of 82.27%. The fourth principal component (RL) had an eigenvalue of 1.43907, explaining 6.54% of the variance, and a cumulative variance of 88.81%. Subsequent principal components each explained progressively smaller amounts of variance: SDW had an eigenvalue of 0.85362 (3.88% variance), CHL a had 0.62123 (2.82%), CHL b had 0.34662 (1.58%), and Cart had 0.18303 (0.83%). $H_2O_2$, MDA, CAT, APX, GST, POD, and GSH each contributed less than 1% to the total variance, with eigenvalues ranging from 0.16026 to 0.00308. The cumulative variance reached 99.92% with GST, and the final principal components POD and GSH brought the total explained variance to 100% (Fig. 7A).

### 3.7.2. Hierarchical Cluster Analysis

The analysis shows that the variables CHL a and TTSH are highly similar with a similarity value of 5.51254, indicating they cluster together early in the hierarchy. RB and RL also cluster together with a similarity of 8.62301. CAT and PAL form a cluster at a similarity value of 9.05876, while SDW clusters with the previously formed cluster containing CHL a and TTSH at 9.61535. The antioxidant enzymes $H_2O_2$ and MDA show a high degree of similarity, clustering at 11.89205. CHL b clusters with the SDW group at 12.85152, and variables such as SB and NPT form a cluster at 14.30594. The Cart clusters at 14.62988, and APX and another cluster at 14.73014. GST joint a cluster at 16.4533, and GSH clusters at 17.32998. POD and a pre-existing cluster form at 18.2793. SL joins a cluster at 18.94141. Late in the analysis, proline (Pro) and TPC cluster together at 28.4473, followed by the addition of other clusters as the similarity threshold increases. AsA is notably isolated, clustering at a high similarity value of 39.42985, indicating it joins the main cluster hierarchy much later, reflecting its distinct behavior compared to other variables. Finally, the remaining clusters form at higher similarity values, with the most distant clustering occurring for PT at 60.67839, illustrating its unique variance. The hierarchy ultimately converges at a similarity of 36.93089 and 39.32161 for the final clusters before all variables are grouped in the dendrogram at the end of the analysis (Fig. 7B).

### 3.7.3. Pearson Correlation Analysis

Strong positive correlations were observed between SB and SL ($r = 0.75606$), SB and SDW ($r = 0.84047$), and SB and TTSH ($r = 0.66682$), indicating these variables are closely related. Similarly, SDW showed a high positive correlation with TTSH ($r = 0.88304$), and CHL a was strongly correlated with TTSH ($r = 0.92252$). Other notable positive correlations include NPT and SB (r = 0.79892) as well as NPT and TTSH (r = 0.61884). Conversely, strong negative correlations were found between SB and $H_2O_2$ (r = -0.89297) and GST and SB (r = -0.86298). $H_2O_2$ was also negatively correlated with SDW (r = -0.82056) and CHL a (r = -0.78955). Additionally, MDA showed strong negative correlations with CHL a (r = -0.84489) and SDW (r = -0.70654), while GST was negatively correlated with many variables, including SDW (r = -0.89307) and CHL a (r = -0.81223). Moderate positive correlations were identified between SB and RB (r = 0.67908), SB and Cart (r = 0.68052), and CHL a and Cart (r = 0.83154). NPT showed moderate positive correlations with several variables, including CHL a (r = 0.68048) and CHL b (r = 0.78606). Moderate negative correlations included SB and CAT (r = -0.56776) and SL and $H_2O_2$ (r = -0.82761) (Fig. 7C).

## 4. Discussion

Due to their widespread application and distribution in agroecosystems, pesticides significantly threaten non-target crops, leading to heightened ecological health risks (Kenko et al., 2023). In the Benchicao region of Medea, Algeria, the prevalent pesticides CYP and HML coexist in viticulture and arboriculture fields. Existing research has demonstrated that CYP disrupts plant growth and influences oxidative responses (Ramzan et al., 2022). Moreover, recent studies emphasize the positive impact of Si on plant responses to xenobiotic stress (Ahire et al., 2021; Asgher et al., 2024). However, information regarding the potential of Si to alleviate combined stresses induced by CYP and HML is limited. For the first time, this study introduces the mitigating role of Si on the growth of tomato seedlings exposed to both insecticide and fungicide stresses.

The results from the fundamental physiological parameters of tomato seedlings clearly indicate substantial growth inhibition, accompanied by visible phytotoxic signs like chlorosis, resulting from the concurrent exposure to CYP and HML, compared to control seedlings. The growth inhibition in seedlings exposed to combined stresses is more pronounced than in those exposed to either CYP or HML individually as shown in Fig. 1. Both shoot and root fresh weights, as well as lengths, exhibit a significant reduction in seedlings subjected to single stress compared to control seedlings. Biomass accumulation in terms of seedlings dry weight showed a similar trend following exposure to CYP and HML pesticides. Notably, these tested pesticides exhibit inhibitory effects on the growth and development of non-target crops, leading to diminished biomass yield (Mahapatra et al., 2019). Literature suggests that CYP may accumulate in the soil, inducing oxidative damage to leaves and impacting the growth of H. annuus and *B. juncea* plants (Ramzan et al., 2022). Intriguingly, each of these growth parameters is significantly lower in seedlings exposed to combined stresses than in seedlings exposed to either contaminant individually, implying a synergistic effect in inhibiting shoot and root growth. This suggests a higher level of damage resulting from the combination of CYP and HML compared to the damage caused by each pesticide individually. The mechanism underlying this phenomenon necessitates further investigation. Similarly, (Touzout et al., 2021a) observed that co-exposure to IMI and Cd significantly worsened the phytotoxicity of tomatoes compared to single exposure. Additionally, Alves et al. (2023) reported that a mixture of glyphosate, 2,4-D, imidacloprid, and iprodione pesticides displayed a synergistic phytotoxic and cytogenotoxic effect on *Allium cepa*. However, the application of Si from an external source mitigated the detrimental effects of both single and combined pesticide exposures as demonstrated in Fig. 1. Compared to individual CYP and HML treatments and the combined treatment, the external application of Si with CYP and/or HML positively influenced both shoot and root growth in tomato seedlings, alleviating pesticide toxicity as indicated in Fig. 1. Previous studies have highlighted the capability of Si in mitigating pesticide and salt-induced phytotoxicity in *S. lycopersicum* by reducing oxidative stress and enhancing pollutant tolerance (Touzout, 2023). Similar positive outcomes have been observed in various plant species, such as promoting plant growth in rice under butachlor toxicity by using Si (Tripthi et al., 2020). This suggests that the application of Si in stressful environments can mitigate the toxicological impact of pesticide stress on plant growth. A study on *S. lycopersicum* by Soares et al., (2021) suggested that Si effectively alleviates the phytotoxic effects of glyphosate herbicides. Based on these findings, it is hypothesized that combined exposure to CYP and HML induces a more pronounced phytotoxic effect than individual exposure, and Si supplementation further alleviates the phytotoxic impact of combined pesticides on tomato growth.

Photosynthetic pigments are commonly utilized as indicators to evaluate the impact of contaminants on plants' phytotoxicity and oxidative damage (Liu et al., 2019). In this investigation, the exposure of tomato seedlings to CYP and HML resulted in a reduction in chlorophyll pigments, with co-exposure demonstrating less severe effects compared to individual exposures, as shown in Fig. 2. Similar trends have been observed in previous studies where pesticide exposure led to a decrease in chlorophyll contents in wheat (Li et al., 2023) and brinjal crop (Singh et al., 2023). This study emphasizes that individual exposure to CYP and HML induced damage to the photosynthetic machinery in tomatoes. At the same time, co-exposure resulted in less severe damage to the pigments, as indicated in Fig. 2. Correspondingly, adverse effects on chlorophyll contents were reported in Arabidopsis thaliana when exposed to silver nanoparticles and diclofop-methyl concurrently (Li et al., 2018). The observed reduction in this study may be attributed to CYP and HML inhibiting chlorophyll biosynthesis enzymes, such as protochlorophyllide reductase and δ-aminolevulinic acid dehydratase activities. Additionally, it might involve modifying chlorophyll molecule structures through the replacement of $Mg^{2+}$ with xenobiotics, leading to induced ROS generation and severe oxidative injury, including lipid peroxidation (Sharma et al., 2019; Touzout et al., 2021b).

Earlier studies have indicated that pesticides can induce chlorophyll degradation in plants. For instance, (Fatma et al., 2018) reported a significant decrease in photosynthetic pigment content in *Vigna radiata* following pesticide application. The phytotoxic effects and potential agricultural security hazards of co-exposure to polyethylene microplastics and cadmium on the photosynthetic performance of *Zea* *mays* have also been documented (Li et al., 2023). Conversely, when seedlings were treated with Si concurrently with CYP and/or HML exposure, a lesser inhibition in chlorophyll a and b contents was observed compared to the control. The addition of Si resulted in a notable increase in chlorophyll content compared to CYP and/or HML treatments without Si as can be seen from Fig. 2B, indicating a positive role of Si in chlorophyll synthesis. Similarly, a study suggested that Si in hydroponic culture positively influenced chlorophyll content in tomato seedlings, consequently promoting seedling growth (Touzout, 2023). The findings suggest that exogenous Si could mitigate the pesticide-induced reduction in seedling photosynthetic pigments, aligning with prior studies in the literature (Soares et al., 2021; Tripthi et al., 2020).

Carotenoids, serving as accessory light-harvesting and structural pigments in addition to their role in photosynthesis, play a photoprotective function by quenching harmful free radicals as antioxidants (Gill and Tuteja, 2010). In this study, carotenoid content decreased concurrently with the reduction in chlorophyll under pesticide stress as shown in Fig. 2. The significant decrease in carotenoid content under single and combined exposures indicates the alteration of photosystem II (PSII), affecting photosynthetic activity (Gill and Tuteja, 2010). In this study, carotenoid content decreased concurrently with the reduction in chlorophyll under pesticide stress as can be seen from Fig. 2. The significant decrease in carotenoid content under single and combined exposures indicates the alteration of PSII, affecting photosynthetic activity (Singh et al., 2023). Similarly, (Li et al., 2023) reported a decrease in carotenoid content in wheat following difenoconazole exposure. Nevertheless, exogenous Si significantly alleviated the pesticide-induced decrease in carotenoid levels in tomato seedlings as shown in Fig. 3C. All Si-supplemented treatments showed a significant increase in carotenoid content compared to the control. This could be attributed to Si enhancing the efficient utilization of plant light energy in photosystem II, thereby reducing the phytotoxic effects induced by CYP and/or HML on seedlings (Tripthi et al., 2020).

Additionally, Si might reduce oxidative stress triggered by pollutants, indirectly safeguarding seedlings from pesticide exposure.

Upon exposure to xenobiotics, an accumulation of reactive oxygen species (ROS) beyond normal levels induces oxidative stress, which can be phytotoxic and detrimental to the cellular system (Soares et al., 2019a). Malondialdehyde (MDA), a by-product of lipid peroxidation, is commonly used as an oxidative stress biomarker in plants (Mittler, 2017). In this study, $H_2O_2$ quantification was utilized to assess how pesticides influenced ROS generation. The results indicate an increase in $H_2O_2$ content under the stressful conditions of CYP and HML compared to the control as can be seen from Fig. 2A. A previous study reported a marked increase in $H_2O_2$ generation under the combined effect of heavy metals and pesticides in tomatoes (Ahammed et al., 2013). However, the content of $H_2O_2$ was significantly reduced in the stressed seedlings after Si application, shown in Fig. 1A, suggesting that Si application regulated ROS homeostasis under pesticide stress. Similarly, the MDA content significantly increased after single exposure to CYP and/or HML, indicating the impact of pesticides on lipid peroxidation in seedling leaves. This aligns with previous studies reporting oxidative stress induced by pesticides in melon (Wu et al., 2023), wheat (Li et al., 2023), *Spirulina* (Touzout et al., 2023) and maize (Mehrian et al., 2023). Interestingly, co-contamination of CYP and HML did not exacerbate the degree of lipid peroxidation, possibly due to the antagonistic effect of the pesticide mixture on lipid peroxidation. In agreement, (Touzout et al., 2021a) reported that exposure to imidacloprid (IMI) and/or cadmium (Cd) induced lipid peroxidation in tomato leaves. However, interactive phytotoxic effects of contaminants have rarely been reported in plants.

It is crucial to note that the underlying mechanism of co-contamination remains unclear. Nevertheless, Si demonstrated a beneficial role by causing a significant reduction in lipid peroxidation, as shown in Fig. 3A. The alleviatory role of Si is evident in reversing the impact of pesticides on the lipid peroxidation of tomato seedling leaves. A study by Soares et al. (2021) similarly demonstrated that Si amendment reduced the degree of oxidative damage in tomato plants under glyphosate stress. Exogenous application of Si also reduced lipid peroxidation in rice (Tripthi et al., 2020). Plant growth regulators, such as Epigallocatechin-3-Gallate (flavonoid), have been reported to alleviate glyphosate and carbendazim phytotoxicity in *Cucumis melo* (Wu et al., 2023).

The phytotoxic mechanism of pesticides is associated with oxidative stress, leading to alterations in antioxidant enzymes and disturbances in redox status (Gill and Tuteja, 2010; Mittler, 2017). In this study, a significant enhancement in CAT and APX activities was observed between the control and pesticide-exposed groups, potentially a result of excessive $H_2O_2$ accumulation directly activating antioxidant enzymes (Gill and Tuteja, 2010; Mittler, 2017). This suggests that antioxidant enzyme activities were stimulated to adapt to the oxidative stress induced by CYP and/or HML exposures. Notably, the oxidative stress induced by the HML fungicide was stronger, as indicated by significantly more pronounced stimulation of CAT and APX activities compared to the CYP insecticide exposed group as demonstrated in Figs. 4A and 4B. Importantly, it can be seen from Figs. 4A and 4B that pesticides co-exposure did not further enhance the oxidative stress of tomato seedlings exposed to both pesticides individually. Thus, the findings revealed that individual and combined CYP and HML exposures activated the antioxidant defense system in tomato leaves. Similarly, Li et al. (2023) reported that the antioxidant enzyme activities of wheat were enhanced under difenoconazole (DFN) fungicide exposure. However, when the stressed seedlings were amended with Si, an even greater decrease in CAT and APX enzyme activities was observed in Figs. 4A and 4B. Reduced antioxidant enzyme activities in Si-amended seedlings under pesticide toxicity indicated less accumulation of $H_2O_2$ as shown in Fig. 3B. This finding was in line with our previous study, where Si supplying to salt and/or voliam insecticide-stressed seedlings was found to decrease the activity of CAT in tomato leaves (Touzout, 2023). Interestingly, when CYP and HML co-exposed seedlings were amended with Si, an even greater increase in CAT and APX enzyme activities was observed as shown in Figs. 4A and 4B, suggesting improved activity of antioxidant enzymes under combined CYP and HML exposures. In line with our results, Tripthi et al. (2020) stated that the application of Si promoted the expression of antioxidant enzyme genes in rice plants following herbicide exposure.

GST plays a crucial role in Phase II of xenobiotic detoxification, conjugating pesticides with reduced glutathione to form non-toxic and water-soluble glutathione S-conjugates (Zhang and Yang, 2021). In this study, the results in Fig. 4C show that treatment with CYP and HML significantly induced the activity of GST. Moreover, co-exposure of CYP and HML further induced the catalytic activity of GST, suggesting their synergistic effect on the detoxification system as indicated in Fig. 4C. This is attributed to GST's involvement in the biotransformation and metabolism of CYP and HML (Yu et al., 2023). Similarly, a significant increase in GST activity was reported in difenoconazole-stressed wheat plants (Li et al., 2023). Seedlings exposed to pesticides and supplied with Si exhibited lower GST activity than those exposed to CYP and/or HML without Si. Plant growth regulators, such as melatonin, have been reported to improve the degradation of chlorothalonil residue in tomato leaves by enhancing the transcripts and enzyme activity levels of GST (Peng et al., 2023b).

Peroxidase is an important defense enzyme in plant antioxidant systems (Liu et al., 2019). In this study, a significant enhancement of POD activity was observed in tomatoes exposed to cypermethrin, either individually or in a mixture with hymexazol (Fig. 4D). With the persistent generation of $H_2O_2$ in seedling leaves, POD activity also increased to mitigate its toxic effects. The improved catalytic activity of POD suggests that CYP and HML can accelerate lipid peroxidation in tomatoes, consistent with the evident growth effects and phytotoxicity observed when seedlings were exposed to pesticides as shown in Fig. 1. POD activity has been reported to be induced in tomato seedlings exposed to deltamethrin and/or cadmium toxicity (Touzout et al., 2021b). The heightened POD activity reinforces the detoxification process. Furthermore, the increased POD activity in CYP and HML co-exposed seedlings due to Si supplementation might play a vital role in the metabolism of CYP and HML, a crucial step in detoxification reactions (Peng et al., 2023b; Yu et al., 2023). Similarly, Si's upregulation in POD activity has been reported in tomatoes under salt and voliam stress (Touzout, 2023), though this differs from the present study.

Additionally, it is important to emphasize that limited knowledge is available regarding eco-friendly solutions, such as Si supplementation, to enhance pesticide metabolism. Indeed, this study represents the initial attempt to provide conclusive evidence that Si application mitigates cypermethrin and hymexazol-induced phytotoxicity and oxidative injury by improving the detoxification system and eliminating ROS in crops.

Sulfur-containing compounds, particularly low molecular weight thiols, act as essential redox modulators, with the ubiquitous tripeptide glutathione (GSH) playing a key role in xenobiotic detoxification (Zagorchev et al., 2013). In this study, GSH content noticeably decreased in response to CYP and/or HML exposures, indicating impairment of GSH recycling and redox state as shown in Fig. 5C. It has been previously suggested that the reduction in GSH content could be a consequence of $H_2O_2$ over-generation (Noctor et al., 2012). The results obtained in this study, shown in Fig. 3A, align with this notion, as MDA specifically accumulated in leaves in response to CYP and/or HML. Moreover, the decrease in GSH content observed in tomatoes under pesticide stress leads to a lower ability for $H_2O_2$ detoxification and, consequently, higher phytotoxicity of pesticides on tomatoes (Soares et al., 2019c). Yu et al., (2023) reported that glutathione contents were altered by the application of chlorothalonil, suggesting the role of glutathione-mediated redox homeostasis in the biotransformation of insecticide in tomato leaves. Additionally, Li et al. (2023) explored that exposure to difenoconazole caused a remarkable promotion of glutathione metabolism in wheat. This result suggests that changes in GSH content might regulate the degradation of CYP and HML in tomatoes by improving GST enzyme (Yu et al., 2023). Conversely, Fig. 4A shows a noticeable increase with Si administration compared to the counterpart CYP and/or HML treatments. The decline in GSH triggered by pesticide exposures was reversed by Si amendment. Likewise, Si amendment's alleviatory role on the GSH redox state levels was reported in rice seedlings against butachlor stress (Tripthi et al., 2020). Furthermore, thiol profiles showed a similar trend to GSH content, as can be seen in Figs. 5A, 5B, and 5D, and a decrease in thiol profile contents (total thiol content (TTSH), protein thiol content (PT), and non-protein thiol (NPT)) certainly affects defense/detoxification pathways. More importantly, the application of Si on CYP and/or HML-stressed seedlings significantly increased thiol profile contents, possibly by stimulating transcription of genes involved in thiol biosynthesis (Yu et al., 2023).  The increases in thiols triggered by Si addition indicate that low-molecular-weight thiols play vital roles in the detoxification of pesticides (Zhang et al., 2017, 2021).

As a dynamic process, secondary metabolism plays a critical role in the adaptation of plants to biotic and abiotic stress (Sharma et al., 2019a). Phenolic compounds, such as flavonoids, can serve as substrates for many enzymes to scavenge ROS in plants and increase pesticide tolerance (Zhang et al., 2022). Figs. 6A and 6B show that PAL activity was significantly induced when exposed to individual pollutants, suggesting that the phenylpropanoid pathway was stimulated by the CYP insecticide and the HML fungicide exposures. In comparison, a notable increase in PAL activity was revealed in Figs. 6A and 6B when exposed to CYP and HML, indicating that the binary mixture of pesticides further induced the phenylpropanoid pathway. Total phenolic content under single and combined exposures was significantly enhanced, suggesting that secondary metabolites were probably stimulated by exposure to CYP, HML, and their combination (Sharma et al., 2019b; Wu et al., 2023). Similarly, Ahammed et al. (2013) reported that enhanced PAL activity was induced in response to HAP and/or Cd-exposed tomatoes, which might affect the respective secondary metabolite synthesis required for active antioxidative protection, supporting the results presented in Figs. 6A and 6B. Furthermore, important changes in PAL activity and total phenolic content were observed under single and combined exposures, indicating that the phenylpropanoid pathway could be stimulated (Sharma et al., 2019). More importantly, Si follow-up treatment on stressed seedlings further increased PAL activity as shown in Figs. 6A and 6B might influence the respective phenolic accumulation required for active ROS scavenging, reflecting the positive effect of Si in biosynthesizing these metabolites. In agreement with our finding, a marked stimulation of phenolic compound content upon exposure to Si indicates its effect on alleviating pesticide-induced phytotoxicity in wheat (Iwaniuk et al., 2022). However, further studies should be carried out to explore the role of Si in the induction of the phenylpropanoid pathway in non-target crops exposed to pesticides.

An alternative way plants respond to pollutants is the accumulation of osmolytes, with proline, a prominent osmolyte, playing a crucial role in mitigating the impact of contaminant exposure (Spormann et al., 2023). Specifically, free proline serves as an osmoprotectant, a stabilizer for macromolecules, and an antioxidant that helps scavenge ROS and inhibit lipid peroxidation (Gill and Tuteja, 2010). The significant elevation in Pro content observed under CYP and/or HML stresses in this study shown in Fig. 6C suggests a favorable influence of pesticides on proline metabolism. This finding aligns with previous research indicating a substantial increase in proline content in tomato leaves following chlorothalonil application (Yu et al., 2023). Additionally, the reported impact on proline metabolism due to acetamiprid, cyromazine, and their mixture in *Vigna unguiculata* corresponds to our observations (Zhang et al., 2022). The augmented Pro accumulation aids in the plant's ability to withstand increased osmotic pressure, ultimately enhancing water absorption by the seedlings (Soares et al., 2019b). Existing literature suggests that pollutants induce oxidative injury and elevate lipid peroxidation, contributing to plant proline accumulation (Zhang et al., 2022). This may explain the increased lipid peroxidation in tomato seedlings exposed to pesticides, as shown in Figs. 3A and 6C. Remarkably, the application of Si to pesticide-stressed seedlings further amplifies proline accumulation compared to individual pesticide treatments, as shown in Fig. 6C, potentially by inducing the transcription of genes and the activity of Δ1-pyrroline-5-carboxylate synthetase involved in proline biosynthesis (Tripthi et al., 2020).

In the present study, the AsA contents of tomato leaves were significantly diminished following exposure to HML alone or in combination with CYP, accompanied by a notable induction of APX activity, as can be seen from Fig. 6D. This suggests that HML and CYP+HML exert potent oxidative effects on tomato seedlings. Furthermore, $H_2O_2$ is dismutated by APX using reduced ascorbate as the electron donor (Foyer and Noctor, 2011), indicating that the AsA–GSH cycle plays a substantial role in antioxidant defense in seedlings. A prior study has indicated that imidacloprid alone and combined with cadmium may reduce AsA content in tomatoes (Touzout et al., 2021a). Additionally, AsA content decreased in *Brassica chinensis* plants exposed to the combined stresses of cadmium and sulfamethazine (Zhou et al., 2021). It is presumed that exposure to CYP, HML, and their combination altered the redox status of AsA, leading to impaired AsA generation (Soares et al., 2019b). Exogenous Si application to seedlings stressed with CYP and/or HML did not induce significant changes, except for the notable decrease in AsA content in seedlings following Si+CYP exposure, as shown in Fig. 6D. Therefore, it is inferred that exposure to CYP, HML, and their combination induced metabolic alterations in tomato seedlings.

Fig. 1 shows that the exogenous application of Si enhances the growth of tomato seedlings under CYP and HML co-exposure. Previous reports have highlighted improvements in the growth of tomato seedlings and increased resistance to pesticides as benefits of Si (Soares et al., 2021; Tripthi et al., 2020). Applying Si facilitates plant crop growth and development under adverse conditions and contributes to abiotic stress tolerance (Arif et al., 2021). Moreover, there is limited knowledge about eco-friendly solutions to reduce pesticide residues for food safety. Thus, a deeper understanding of the role of Si in pesticide degradation can contribute to the development of new detoxification methods to safeguard public health from pesticide residue issues. To the best of the authors' knowledge, this study can be regarded as the first attempt to provide conclusive evidence that exogenous Si supply mitigates CYP and HML-induced growth inhibition and oxidative damage through an improved detoxification system and secondary metabolism in crops.

## 5. Conclusion

This investigation underscores the deleterious consequences of exposing tomato seedlings to CYP and HML, resulting in compromised photosynthetic pigments, heightened oxidative stress, reduced thiols responses, and subsequent hindrance to the detoxification system, ultimately impeding growth. Furthermore, the co-contamination scenario exhibited a more pronounced phytotoxic effect when compared to individual exposures to CYP and HML. The synergy between CYP and HML further intensified the phytotoxic impact on non-target crops within the agroecosystem. Additionally, the application of Si demonstrated its capability to mitigate the adverse effects of CYP and HML on growth and photosynthesis. Si achieves this by enhancing the biotransformation capacity of pesticides and promoting secondary metabolism. The findings suggest that Si supplementation is a promising eco-friendly approach to reduce pesticide residues, contributing to heightened food safety significantly. To comprehensively understand the phytotoxic effects of pesticides on non-target crops and their mitigation by Si, further investigations integrating mutant analyses and omics approaches are recommended.

## CRediT authorship contribution statement

**NT** and **MB**: Conceptualization of the project. **NT**, **HT** and **MB**: Manuscript drafting. **AM, JZ, SD** and **AA**: Contribution to the editing and proofreading of the manuscript draft. All authors approved the final version.

## Availability of Data and Materials

The data and material for the current study are included in this published article.

## Ethics Approval and Consent to Participate

Not applicable.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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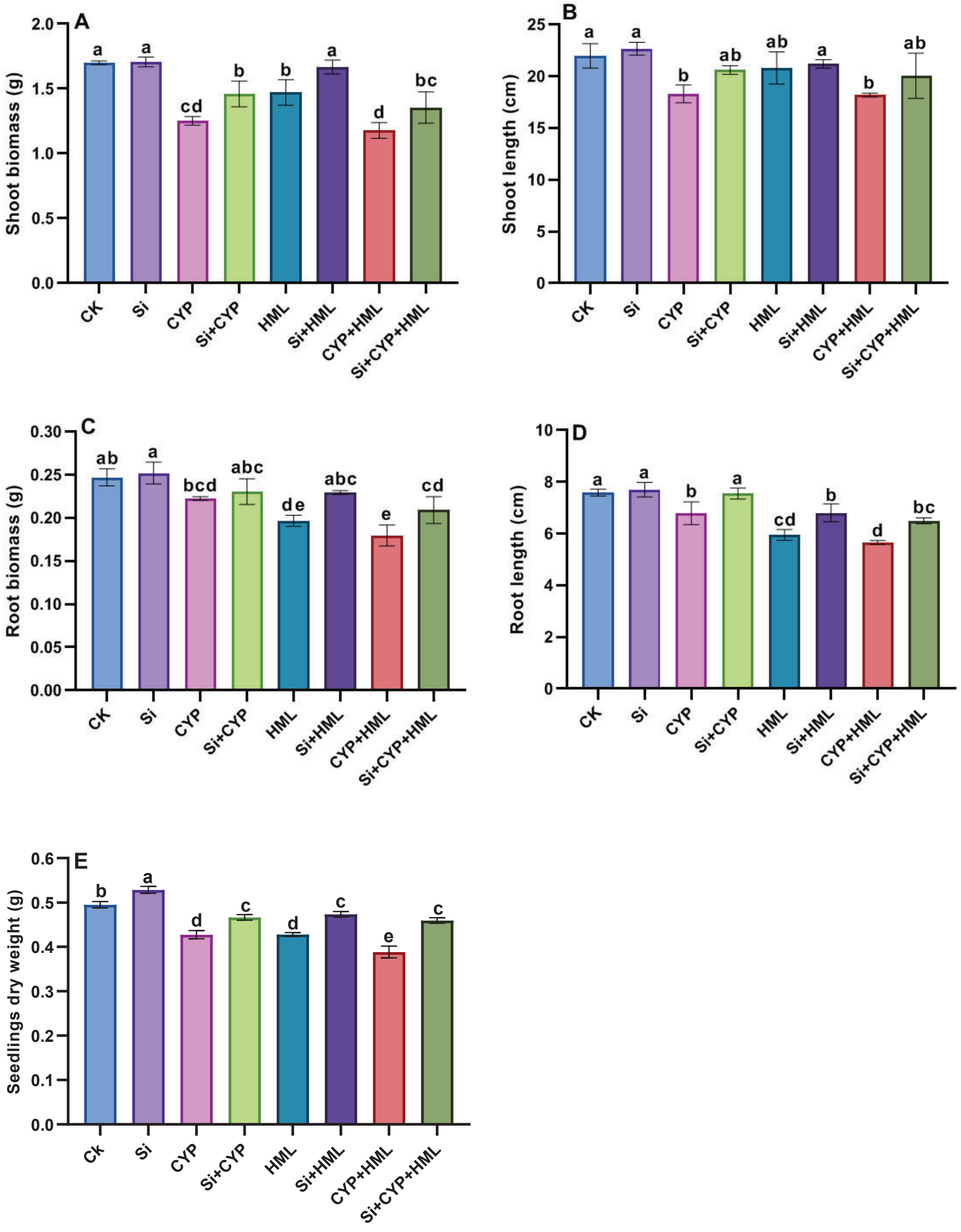

**Fig. 1.** Effect of silicon treatments (CK: Control; Si: Silicon; CYP: Cypermethrin; HML: Hymexazol; Si + CYP: Silicon + Cypermethrin; Si + HML: Silicon + Hymexazol; CYP + HML: Cypermethrin + Hymexazol; Si + CYP + HML: Silicon + Cypermethrin + Hymexazol) on shoot biomass (A), shoot length (B), root biomass (C), root length (D), and seedlings dry weight (E) of tomato seedlings under solitary and combined cypermethrin and hymexazol exposures. Values presented are means with standard deviations (n = 3). Different letters on each bar indicate significant differences according to the Tukey test ($p < 0.05$).

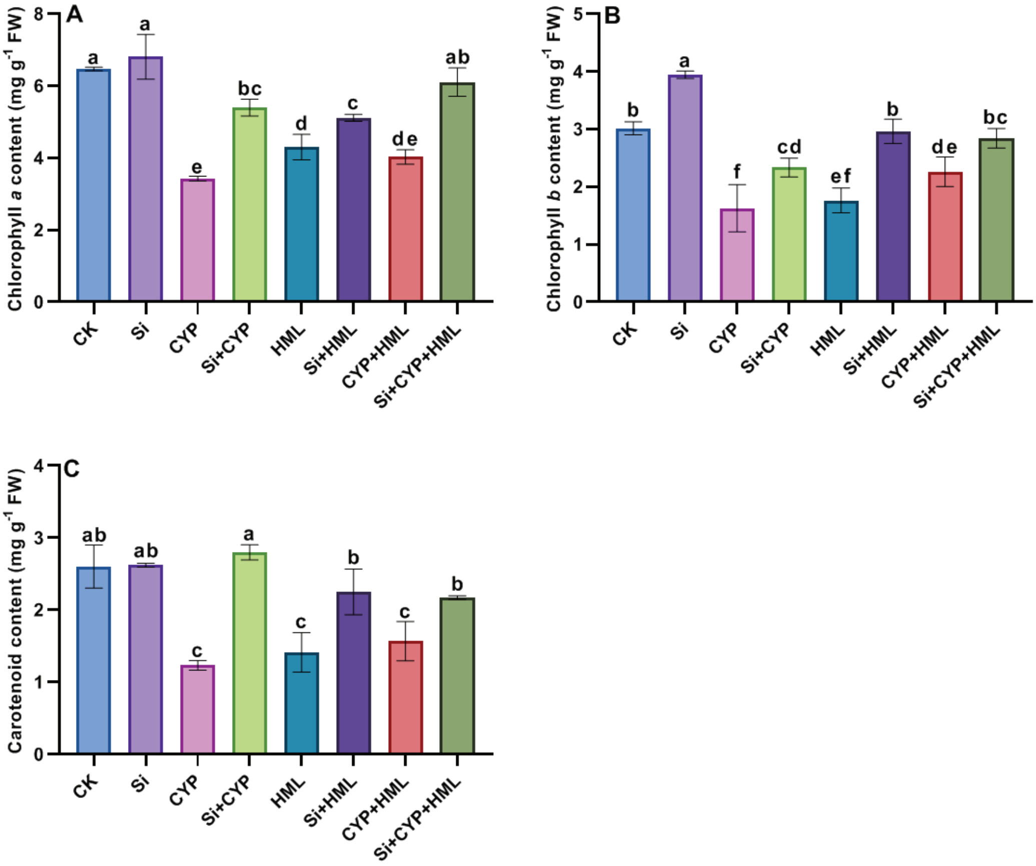

**Fig. 2.** Effect of silicon treatments (CK: Control; Si: Silicon; CYP: Cypermethrin; HML: Hymexazol; Si + CYP: Silicon + Cypermethrin; Si + HML: Silicon + Hymexazol; CYP + HML: Cypermethrin + Hymexazol; Si + CYP + HML: Silicon + Cypermethrin + Hymexazol) on Chlorophyll *a* content (A), Chlorophyll *b* content (B), and Carotenoid content (C) of tomato seedlings under solitary and combined cypermethrin and hymexazol exposures. Values presented are means with standard deviations (n = 3). Different letters on each bar indicate significant differences according to the Tukey test ($p < 0.05$).

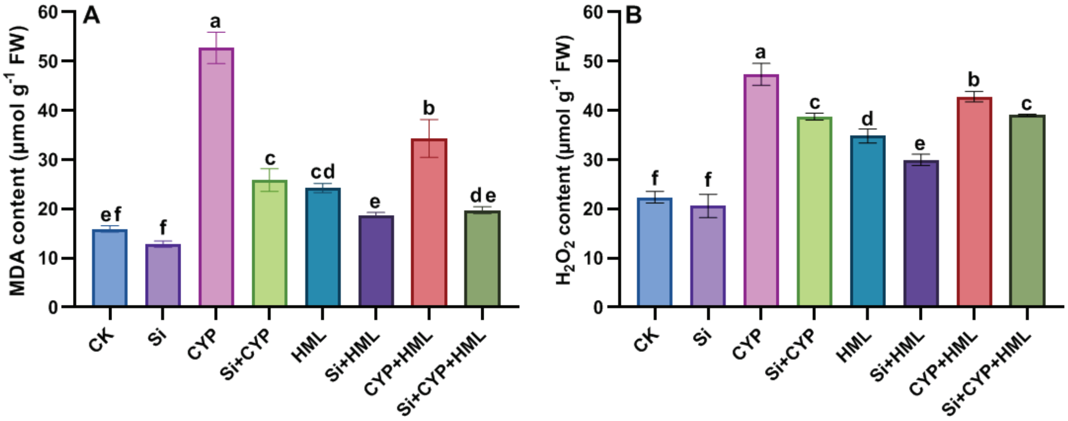

**Fig. 3.** Effect of silicon treatments (CK: Control; Si: Silicon; CYP: Cypermethrin; HML: Hymexazol; Si + CYP: Silicon + Cypermethrin; Si + HML: Silicon + Hymexazol; CYP + HML: Cypermethrin + Hymexazol; Si + CYP + HML: Silicon + Cypermethrin + Hymexazol) on Malondialdehyde content (MDA) (A) and Hydrogen peroxide content ($H_2O_2$) (B) of tomato seedlings under solitary and combined cypermethrin and hymexazol exposures. Values presented are means with standard deviations (n = 3). Different letters on each bar indicate significant differences according to the Tukey test ($p < 0.05$).

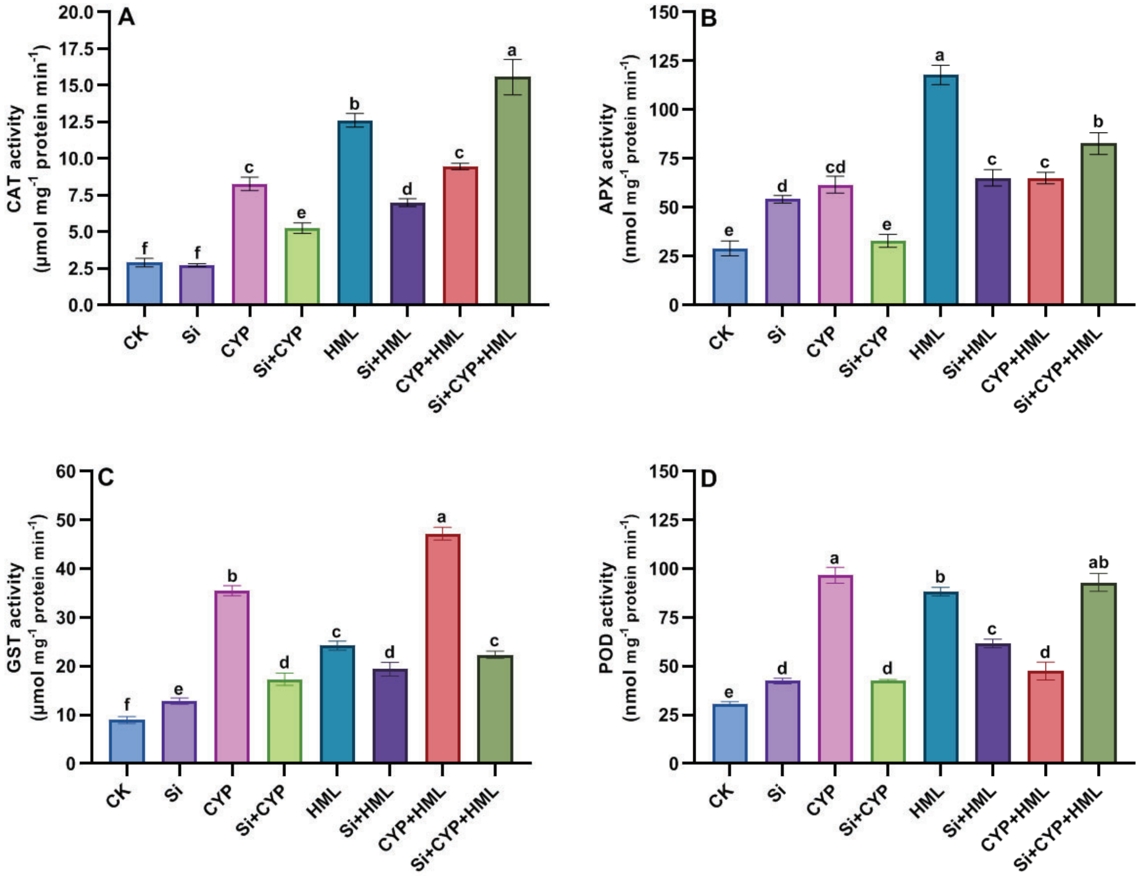

**Fig. 4.** Effect of silicon treatments (CK: Control; Si: Silicon; CYP: Cypermethrin; HML: Hymexazol; Si + CYP: Silicon + Cypermethrin; Si + HML: Silicon + Hymexazol; CYP + HML: Cypermethrin + Hymexazol; Si + CYP + HML: Silicon + Cypermethrin + Hymexazol) on catalase (CAT) (A), ascorbate peroxidase (APX) (B), glutathione S-transferase (GST) (C), peroxidase (POD) (D), and phenylalanine ammonia lyase (PAL) (E) of tomato seedlings under solitary and combined cypermethrin and hymexazol exposures. Values presented are means with standard deviations (n = 3). Different letters on each bar indicate significant differences according to the Tukey test ($p < 0.05$).

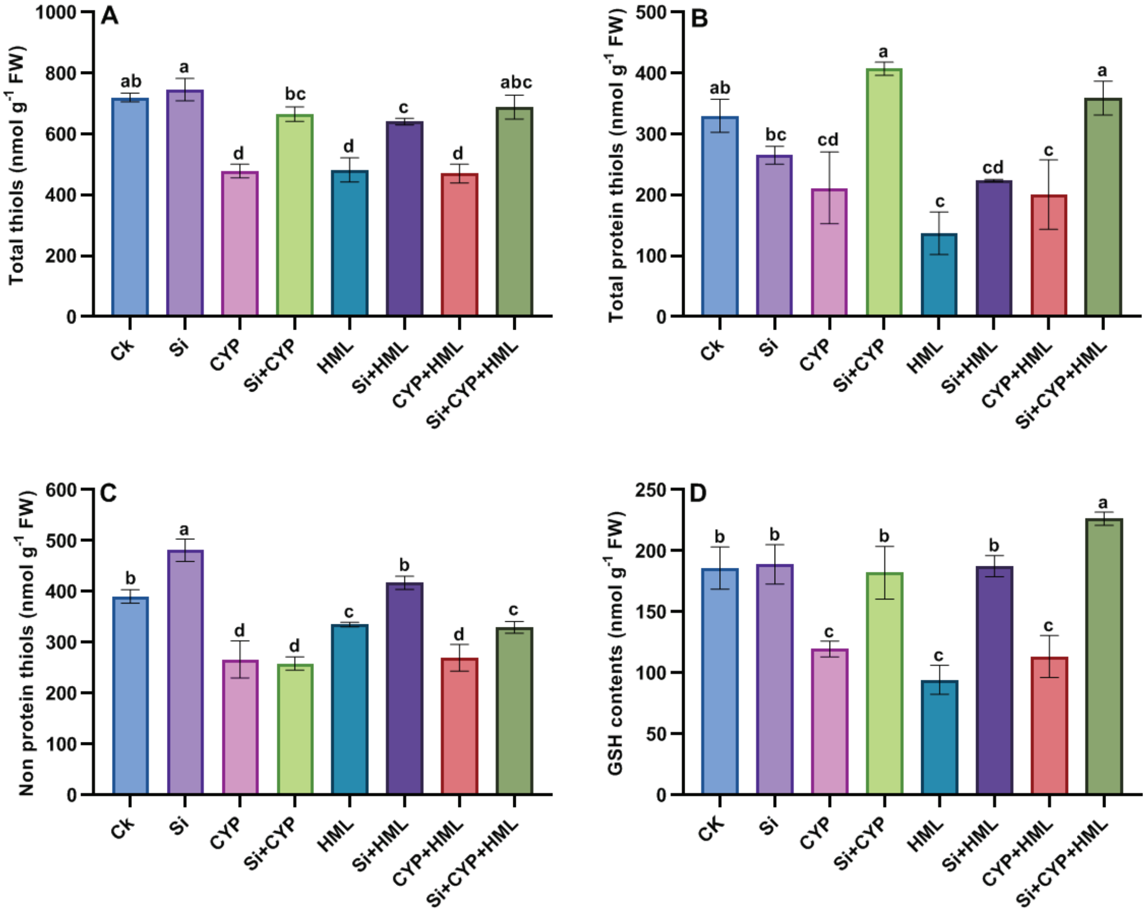

**Fig. 5.** Effect of silicon treatments (CK: Control; Si: Silicon; CYP: Cypermethrin; HML: Hymexazol; Si + CYP: Silicon + Cypermethrin; Si + HML: Silicon + Hymexazol; CYP + HML: Cypermethrin + Hymexazol; Si + CYP + HML: Silicon + Cypermethrin + Hymexazol) on total thiols content (TTSH) (A), protein thiols content (PT) (B), Non protein thiols (NPT) (C), and reduced glutathione (GSH) (D) of tomato seedlings under solitary and combined cypermethrin and hymexazol exposures. Values presented are means with standard deviations (n = 3). Different letters on each bar indicate significant differences according to the Tukey test ($p < 0.05$).

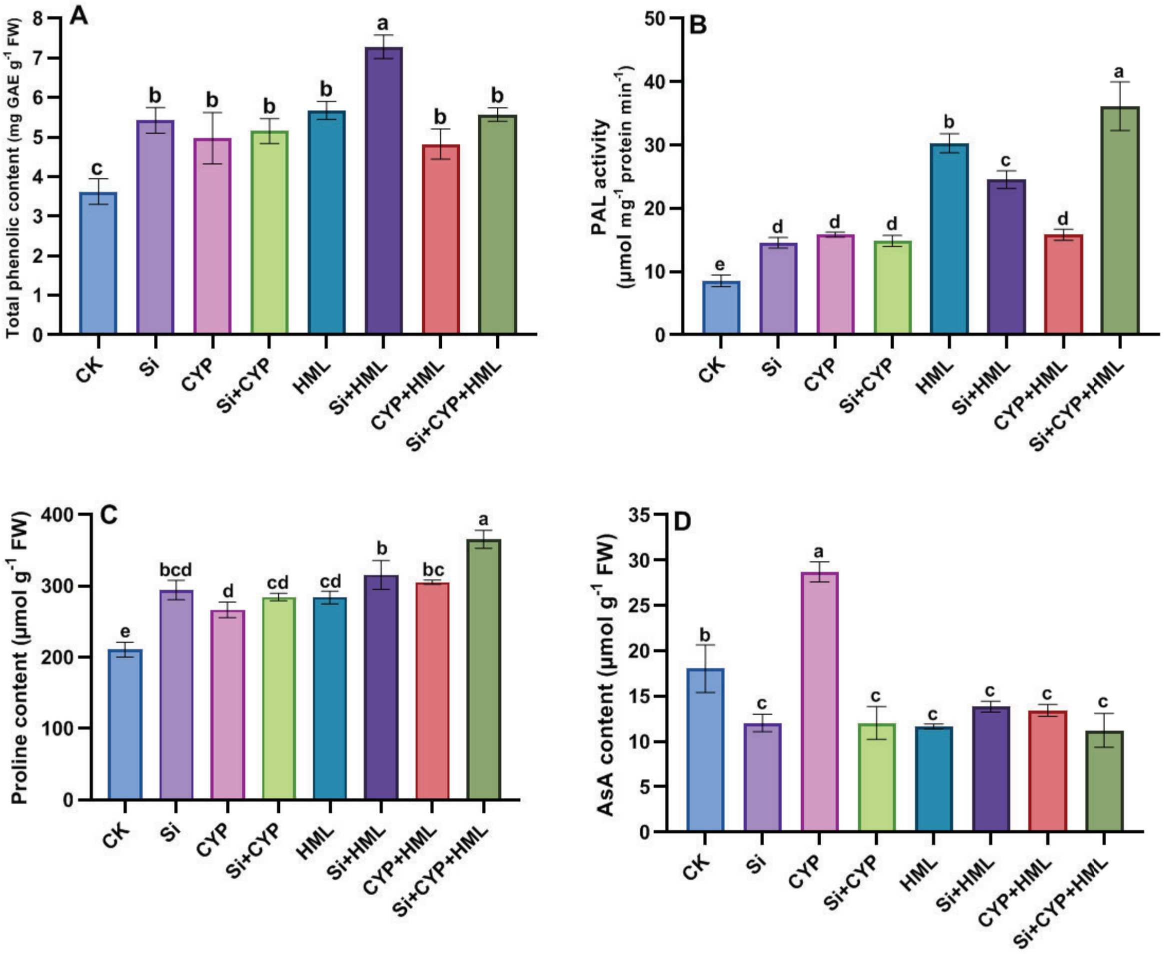

**Fig. 6.** Effect of silicon treatments (CK: Control; Si: Silicon; CYP: Cypermethrin; HML: Hymexazol; Si + CYP: Silicon + Cypermethrin; Si + HML: Silicon + Hymexazol; CYP + HML: Cypermethrin + Hymexazol; Si + CYP + HML: Silicon + Cypermethrin + Hymexazol) on total phenolic content (TPC) (A), proline content (Pro) (B), reduced glutathione (GSH) (C), and reduced ascorbate (AsA) (D) of tomato seedlings under solitary and combined cypermethrin and hymexazol exposures. Values presented are means with standard deviations ($n = 3$). Different letters on each bar indicate significant differences according to the Tukey test ($p < 0.05$).

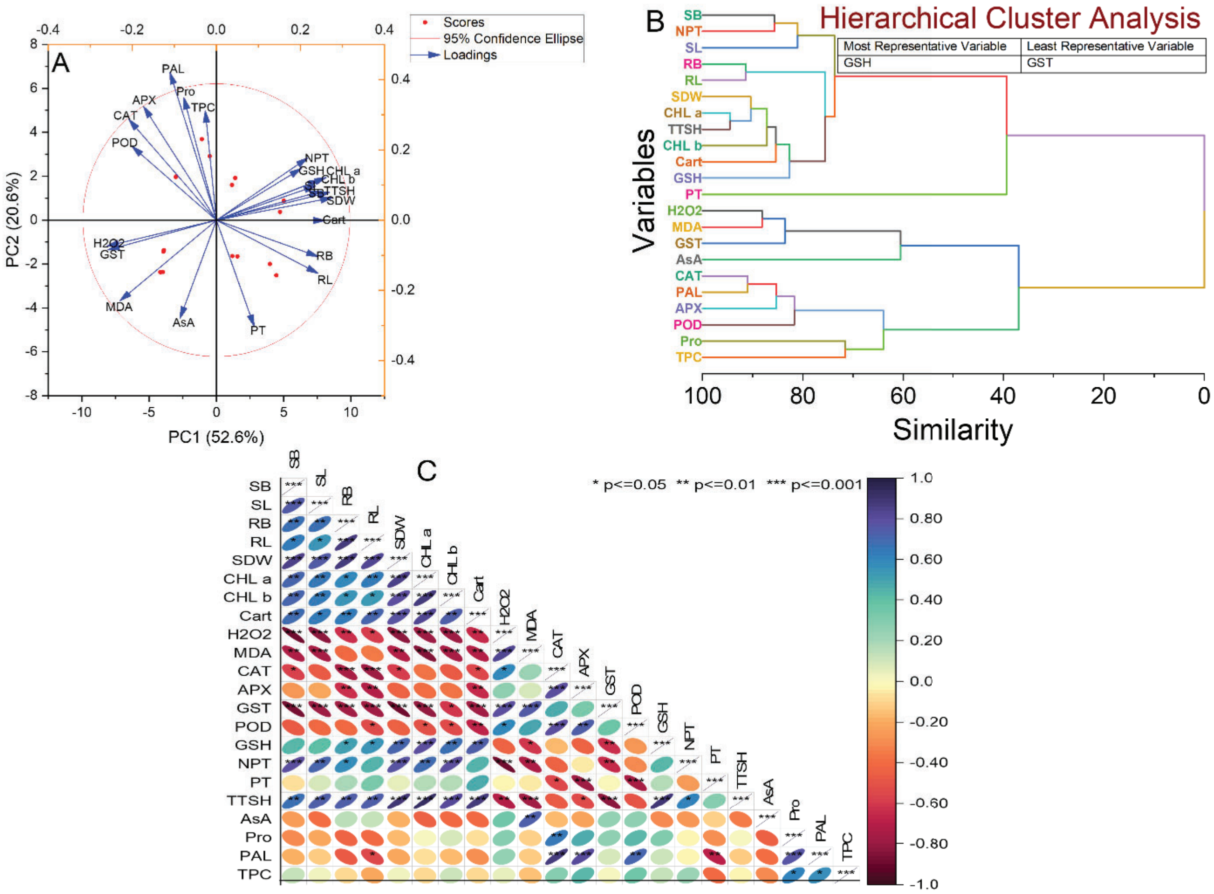

Figure 7. Multivariate analysis to find the relationship between different parameters of tomato plant exposed to Si, CYP and HML treatments. Principal component analysis (A), Hierarchical cluster plot (B) and Pearson correlation analysis (C) of studied attributes of tomato seedlings exposed to Si, CYP and HML treatments.